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S. Umemoto

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A Modified Colorimetric Method of Small Amount Hydrogen Peroxide and its Contents in some Marine Foods

Shigeru UMEMOTO

Abstract: The colorimetric method of hydrogen peroxide determination proposed by MIYAMOTO and YOKOYAMA was modified in order to get sensitivity improvement. Samples were extracted using $ZnSO_4$-NaOH solution system as a deproteinising agent. Quinone-imine dye, the oxidative product of 4-aminoantipyrine and phenol with hydrogen peroxide in the presence of peroxidase was measured at wavelength 505 nm. The modified method was reproducible and simple. The optical density of thc dye produced by 1 ppm hydrogen peroxide solution was found to be 0.2 which corresponded to ca. 8-fold sensitivity of the original method. The determinations were made on several kinds of marine foods such as "Kazunoko" (herring roe), "Kamaboko" (fish cake) and "Sirasu" (boiled whitebait). The results obtained showed that small amount of hydrogen peroxide were found in some foods which were processed without hydrogen peroxide treatment.

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In the past, the volume of residual hydrogen peroxide (abbreviated hereafter as H_2O_2) in food was set at 100 ppm or less for noodles, boiled fish paste and such, and at 30 ppm or less for the other food articles. Recently however, as the carcinogenicity of H_2O_2 became a cause for concern, the Ministry of Health and Welfare revised part of the "Norms and standards for food articles, additives and such" in the law on food hygiene and announced that from October 1980 "the hydrogen peroxide had to be degraded or removed prior to producing the final food products"[1]. To be able to comply with the stricter regulations, one has sought to establish a method for the analysis of trace H_2O_2 in food articles. In the iodine titration method[2,3] adopted in the past as official analysis method, the lower limit of H_2O_2 determination was as high as 10 ppm and the method could therefore not be used to satisfy the above stricter regulations. So, tests and examinations were pursued with regard to the recently reported colorimetric method, the fluorescence method and other methods used to analyze trace H_2O_2. As H_2O_2 which is a simple compound has little reaction specificity, we examined an analytical method involving an oxygen reaction in the analytical operation stages so as to avoid as much as possible any effect upon the measurement values from other oxidative matters which might be admixed to the samples.

Using the principle of cholesterol enzymatic analysis of Allain et al[4], Miyamoto et al[5] reported a H_2O_2 colorimetric determination method (abbreviated as original method) in which the H_2O_2 contained in the food articles is made to react against 4-amino-antipyrine (abbreviated as 4-AA) and phenol under the presence of peroxidase. When

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testing this original method further, we discovered that the color development was stable and that the absorption values were superb in reproducibility. We improved the original method and raised its determination sensitivity (abbreviated as improved method). We present hereunder the improved method as well as H_2O_2 analysis results obtained from a few marine food products.

We used special-grade reagents which were peroxidase (abbreviated as POD) POD, Type II from the Sigma Co., catalase from the Boehringer Manheim Co. The water was glass-distilled water. The concentration of the H_2O_2 standard solution was determined by the iodine method through titration with a N/50 $Na_2S_2O_3$ solution. The lipids were extracted with a mixed chloroform-methanol solution (2:1). The sample foods tested were "kazunoko" (herring roe), fish paste products and "shirasu" (boiled white bait; called "kamaage" and boiled in brine). These food products were H_2O_2-treated at the manufacturing stage, prior to the present stricter regulations about the use of H_2O_2. p. 58

Results and discussion

Raising further the sensitivity of the H_2O_2 colorimetric determination method

We used the original method of Miyamoto et al[5] to prepare a colorimetric solution by adding a large volume of color-development reagent solution (5 mL) to a small volume of reagent extract (0.5 mL).

To raise the sensitivity, we reversed the above mixing proportions and tested the colorimetric method in which a small volume of color-development reagent solution (but of high concentration) is added to a large volume of sample extract. In the original method, the sample is extracted with methanol. If the proportion of sample extract is raised in the colorimetric solution, the methanol concentration increases and the color-development reaction is impeded ass the POD is deactivated. This is why we added various preparatory examinations regarding sample extraction and deproteinization as well as regarding the conditions of colorimetric solution preparation so as to establish the improved method hereunder.

The sample extraction is shown in Fig. 1. We weighed the samples, homogenized together with a $ZnSO_4$ solution, added a NaOH solution, and extracted while deproteinizing. After bringing to a determined volume, we centrifuged and used the supernatant as sample extract. The extraction treatment and the extract preservation were carried out at low temperature. Inasmuch as possible, the colorimetry was performed on the extraction day. Fig. 2 shows the color development treatment and the colorimetric procedure. We added the color-development reagent solution, the POD to the sample extract and performed warm developing after which we measured at a wavelength of 505 nm the absorbance of the quinone imine compound (red-orange color) generated by H_2O_2 and the color development reagent. The absorption peak of the colored matters was seen at 505 nm (Fig. 3). p. 59

Often a transparent extract is obtained with the aforementioned sample extraction treatment, but sometimes a slight cloudiness is also observed in the extract. It happens that at the time when the colorimetric solution undergoes warm color development there is occasionally a formation of light insoluble matters in the color development solution. At this time, one centrifuges (10^4 x g, 10 min) after the warming up, and the supernatant is submitted to colorimetry. The proportion of sample extract is great in the colorimetric fluid, so it is feared that the cloudiness of the extract may cause errors in the absorption values. This is why one corrects the cloudiness of the colorimetric solution. In Fig. 2 the colorimetric solution (B) is used for cloudiness correction.

Fig. 4 shows an example of calibration curve obtained by the p. 60
improved method. A rectilinear proportional relationship is noted (r = 0.09996, n = 38) between the concentrations and the absorption values (ODc) with H_2O_2 solution concentrations of 0.3 - 4.3 ppm. The reproducibility of the regression straight line slope was good too. With a H_2O_2 1 ppm solution, OD indicated about 0.2. The sensitivity was 8 times superior to that of the original method. The dotted line in Fig. 4 is the calibration curve obtained through additional tests of the original method. The practical measurement range with the improved method is 0.1 - 4.3 ppm (for solutions).

We used the above improved method to analyze kazunoko, fish paste products, shirasu and such, and noted no particular obstacles. Table 1 shows the analysis results. Some of the main points modified in the p. 61

original method by the improved method are the following. That is, in the sample extraction based on methanol in the original method a change was made to $ZnSO_4$-NaOH deproteinizing agents. The mixing rate of sample extract and of color-development reagent solution is reversed, so we added a small volume of color development reagent solution (0.3 mL) to a large amount of sample extract (5 mL). The phosphoric acid buffer solution in the colorimetric solution is changed to a Tris-HCl buffer solution and correction of the sample extract cloudiness is performed at colorimetry time. In order to enhance the sensitivity of the original method of Miyamoto et al[5], Ito et al[6] changed the H_2O_2 in the sample methanol extract to stable quinone-imine colored matter. They performed purification by color chromatography, and after concentration treatment and such, proposed a colorimetric determination method of high sensitivity to perform colorimetry. This method is cumbersome for analytical operations but more sensitive than the improved method compared in the present report. One might say that the improved method of the present report is a simple practical colorimetric determination method having a medium sensitivity.

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H_2O_2 addition-recovery tests

If we add H_2O_2 to weighted samples (volume corresponding to about 1-4 ppm in the extract) and consider the H_2O_2 addition-recovery rate at the time the whole operation is carried out from extraction up to colorimetry by the improved method, we see that the rate remains low at 28 - 36% in kazunoko, that it is 52-72% in fish paste products and 54-61%

in shirasu. The recovery rates were thus not too high. If on the other hand we add H_2O_2 (volume corresponding to about 1-3 ppm in the extract) to the sample extract obtained from kazunoko, fish paste products, shirasu and such, perform the colorimetry of this solution and consider the recovery rate, we find the high 96-102% rate. The reasons for the low addition-recovery rate in the aforementioned total analytical course going from extraction to colorimetry are that the H_2O_2 itself is originally unstable and tends to decompose, that it is likely to become degraded when touching organic matters, that H_2O_2-degrading enzymes may be contained in unheated samples (for instance kazunoko) and that these factors may have had some action at homogenizing and extracting times. Whatever the reasons might be, enhancing the addition-recovery rate remains an important question to study. p. 62

In the homogenization of H_2O_2 solutions (no organic matters contained), we noticed almost no H_2O_2 destruction. No H_2O_2 was detected with the improved method even when homogenizing the extracting agent. In the $ZnSO_4$-NaOH system, there was a very large production of white precipitation but no H_2O_2 loss was observed in the centrifuged supernatant. The weight of anhydrous matter in the above white precipitation was not more than 1.3% of the system.

Stability of H_2O_2 in the extract
and reproducibility of the measurement values

Fig. 5 shows the results obtained by examining the changes in H_2O_2

concentration when preserving at low temperature with crushed ice sample solutions prepared by adding H_2O_2 to extract obtained from shirasu. The H_2O_2 is kept in a relatively stable fashion in the extract. It dropped by several % in 6 hours and by about 10% in 24 hours. It is therefore desirable to perform colorimetry on the very day of the extraction. The color of H_2O_2 developed by 4-AA-phenol shows virtually no change in absorbance during 4-hour preservation at room temperature. It was extremely stable. The reproducibility of the absorption values based on the color development of shirasu extract showed for 10 measurement values a variation coefficient of 2.3%, and a high reproducibility was shown. p. 63

We mixed well and homogenized shirasu samples. After the three separate operations of weighing, extracting and colorimetry, we measured the H_2O_2. Table 2 shows the results obtained when observing the reproducibility of the measured values. Eight measurements yielded H_2O_2 3.4 - 3.9 ppm and the variation coefficient was 5.5%. The reproducibility of the measurement values was judged to be relatively high. The reason for choosing shirasu samples is that it is easily possible to homogenize the samples prior to weighing as the mixing is simple.

Examining the explanations for the H_2O_2 readings

At the time we developed and performed the colorimetry of the sample extracts, we also performed similarly the development and colorimetry of sample extract supplemented with catalase. The catalase-treated extract

showed noticeably low H_2O_2 values (Table 3). These results show that the readings are values originating from peroxides which are easily degraded and lost due to catalase.

There was a feeling of oil burn smell in the kazunoko showing high H_2O_2 values. We extracted the lipids from the kazunoko and shirasu with a mixed chloroform-methanol solution (2:1). We washed these lipids with the extraction agents of the aforementioned improved method (supernatant of the mixed $ZnSO_4$-NaOH solution) and obtained a wash. As we developed this wash with 4-AA-phenol color development reagents, treating this wash as we treated sample extracts, we noted a red-orange color similar to that obtained with H_2O_2. The absorption curve of the development solution was likewise very similar to that of H_2O_2 (Fig. 6). p. 64

We left standing lipids samples extracted from kazunoko for about 3 weeks at room temperature and measured the POV as well as the developed color of the lipid wash (Table 4). We noted an increasing POV rise in the lipids and an increased development value in the lipid wash. The color development values were low with regard to catalase-treated lipid wash. The results in Fig. 6, Tables 3,4 show that values originating from lipid peroxides are included in the measurement values based on the 4-AA-phenol colorimetric method.

As Table 1 shows, even marine food products not treated with H_2O_2 exhibit 0,2 - several ppm values, the H_2O_2 not being limited to ND. A type of lactobacillus is reported to produce H_2O_2[7]. Food products may

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contain naturally 0.2 - several ppm of H_2O_2. The H_2O_2 background level in food is something which remains to be examined.

To obtain the samples needed for this study we received the kindest cooperation from the Kokkaido Prefectural Central Marine Experimental Station, from the Prefectural Kushiro Marine Experimental Station, from the processors of the Kokkaido Fisheries Processing United Association and Alliance, from the Yamada Fisheries Research Station, the Iwauchi Town Fisheries Research Center, the Ibaraki Prefectural Marine Experimental Station, the Aji-no Hamafuji Co., the Suzuhiro Kamaboko Industries, the Shizuoka Prefectural Marine Experimental Station and the Wakayama Prefectural Fisheries Promotion Experimental Station. We are grateful to these institutions.

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Reagents

4% $ZnSO_4$ solution

1 N NaOH solution

antiform silicone (Toshiba silicone TSA 737)

extractant: supernatant of 4% $ZnSO_4$ solution + 1 N NaOH solution (10 : 4)

Extraction

Sample (10g)

+4% $ZnSO_4$ solution 20 ml

homogenize

+1 N NaOH solution 8 ml

transfer the homogenate into

a graduated cylinder (50 or 100 ml)

+ antiform silicone, few drops

make up to 50 or 100 ml by adding the extractant

centrifuge, 6,000 × g, 20 min

Sup

Sample extract

Cold reagents are used and the extraction are carried out at low temperature (below 10°C). The obtained extracts are kept cool.

Fig. 1. Reagents and extraction procedures of sample for hydrogen peroxide determination by the modified method.

Reagents

4-aminoantipyrine (4-AA) solution 40 μmol/ml, keep cool

phenol solution 400 μmol/ml, store in drak place

Tris (1.5 M)-HCl buffer solution (Tris buffer), pH 7.8

peroxidase (POD) solution 500 PU/ml, keep cool

(Sigma POD Type II was diluted with water)

reagent mixture for color development, prepare fresh before use

4-AA solution	1 vol
phenol solution	1 vol
Tris buffer	4 vol

Assay system

(A) for color development

Pipette successively	Reference	Sample
Sample extract	— ml	5.00 ml
Extractant	5.00	—
Reagent mixture	0.30	0.30
POD	0.05	0.05

(B) for turbidity correction

Pipette successively	Reference	Sample
Sample extract	— ml	5.00 ml
Extractant	5.00	—
Reagent mixture	0.30	0.30
Water	0.05	0.05

Incubation for color development: 37℃, 15 min. When insoluble matter occurs in the assay solutions, use supernatant of the incubated assay solutions for colorimetry.

Optical density measurement: wavelength 505 nm, light path 1 cm.

Optical density of the developed color (OD_C)

$OD_C = OD_A - OD_B$

where OD_A : optical density of (A)

OD_B : optical density of (B)

Fig. 2. Reagents and procedures for colorimetry of hydrogen peroxide by the modified method (see also Fig. 1).

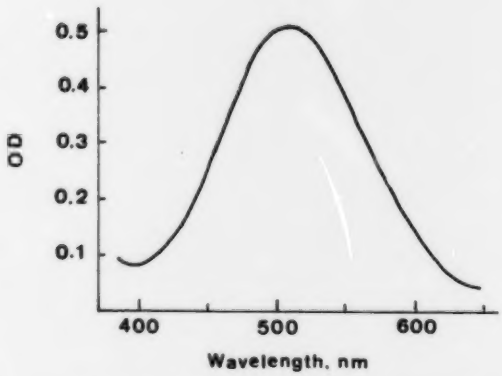

Fig. 3. Absorption curve of hydrogen peroxide-4-aminoantipyrine-phenol system.
Absorption maximum: 505 nm (pinkish red)

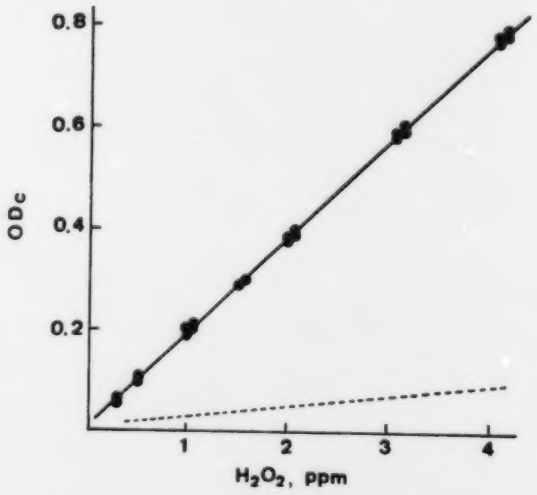

Fig. 4. Calibration curve of hydrogen peroxide by the modified method (see also Fig. 2).
Abscissa: H_2O_2 $\mu g/ml$ of sample extract
Solid line: The modified method
Dotted line: The original method

Table 1. Hydrogen peroxide contents in several kinds of marine foods*

Sample	H_2O_2 ppm	Notes
"Kazunoko"		
(Salted herring roe)	0.3	
〃	0.2	
〃	2.0	
〃	2.9	
〃	0.4	
〃	0.9	
"Kamaboko"		
(Fish cake)	ND	steamed
〃	ND	〃
〃	0.7	〃
〃	ND	boiled
〃	ND	〃
〃	0.2	〃
〃	ND	〃
〃	0.2	fried
〃	0.6	〃
〃	0.2	〃
"Frozen Surimi"		
(Frozen minced meat)	ND	minced fish meat of Alaska pollack for "Kamaboko"
〃	ND	〃
〃	0.2	〃
"Sirasu"		
(boiled whitebait)	2.5	
〃	0.9	
〃	3.1	
〃	2.7	

* Samples tested were processed without hydrogen peroxide treatment.

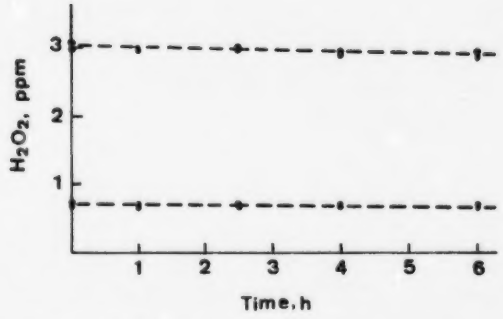

Fig. 5. Changes in hydrogen peroxide concentration of the extracts from "Sirasu" during storage in crushed ice.
The tests were made at two levels of hydrogen peroxide concentration (ca. 3 ppm and 0.7 ppm).

Table 2. Reproducibility by the modified method on hydrogen peroxide content in "Sirasu".

Exp. No.	H_2O_2 ppm
1	3.7
2	3.8
3	3.4
4	3.5
5	3.4
6	3.4
7	3.6
8	3.9
Mean	3.6

Coefficient of variation=5.5%
Sample "Sirasu" were mixed well, then the analyses(weighing, extraction and colorimetry) were carried out separately in eight experiments. Figures show the mean values of triplicate runs of colorimetric measurements in each experiments.

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Table 3. Hydrogen peroxide values obtained with and without catalase treatment of sample extracts from some marine foods.

Sample	H_2O_2 ppm*		Notes
	without catalase treatment	with catalase treatment	Appearance of sample foods and POV of the lipids
"Kazunoko" (salted)	0.9	0.5	normal, yellowish brown, POV: 49
〃	3.7	1.7	discolored, brown, rancid odor, POV: 354
"Kamaboko" (fried)	0.2	ND	normal
"Frozen Surimi"	0.2	ND	normal
"Sirasu" (boiled)	1.6	ND	normal, white, POV: 16
〃	1.4	ND	normal, white, POV: 0

* Figures were expressed as H_2O_2 ppm in the food samples used.

Sample extracts → colorimetry of H_2O_2

Sample extracts → treated with catalase → colorimetry of H_2O_2

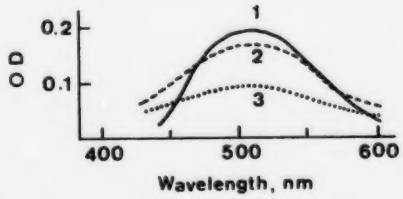

Fig. 6. Absorption curves of the dye produced by lipid washings with 4-aminoantipyrine and phenol in the presence of peroxidase.

Lipids: Extracted from "Kazunoko" and "Sirasu" with chloroform and methanol mixture (2 : 1).

Washing agent: Supernatant of $ZnSO_4$-NaOH solution mixture (same as extractant in Fig. 1).

1: Hydrogen peroxide solution

2: Washings of lipids from "Kazunoko"

3: Washings of lipids from "Sirasu"

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Table 4. Oxidation of "Kazunoko" lipids and color development of the lipid washings with 4-aminoantipyrine and phenol.

Days stored	Peroxide value of lipids	Color development* of lipid washings	Color development* of lipid washings treated with catalase
0	52	0.5 ppm	–
12	103	1.8	0.6 ppm
24	169	3.0	0.3

* Figures were expressed as ppm hydrogen peroxide in the "Kazunoko" tested.

Lipids: Stored at room temperature

Washing agent of lipids: Supernatant of $ZnSO_4$-NaOH solution mixture (same as extractant, in Fig. 1).

Bibliography

1) 厚生省告示 第24号 (昭55.2.20).
2) 梅本 滋・権原和子・天野慶之：食衛誌, 9, 139-146 (1968).
3) 林 敏夫：食品衛生研究, 18, 1050-1053 (1968).
4) C.C. ALLAIN, L.S. POON, C.S.G. CHAN, W. RICHMOND and P.C. FU : *Clin. Chem.*, 20, 470-475 (1974).
5) 宮本邦彦・横山 剛：衛化, 22, 76-80 (1976).
6) Y. ITO, Y. TONOGAI, H. SUZUKI, S. OGAWA, T. YOKOYAMA, T. HASHIZUME, H. SANTO, K. TANAKA, K. NISHIGAKI and M. IWAIDA : *J. Assoc. Off. Anal. Chem.*, 64, 1448-1452 (1981).
7) B. HARONO, M. TAKANO and I. SHIBASAKI : *Annual Report of International Center of Cooperative Research and Development in Microbial Engineering*, 4, 231-243 (1981).

1. Koseisho Kokuji, (Notifications from the Ministry of Health and Welfare) No. 24, Feb. 20, 1980.

2. Shigeru UMEMOTO, Kazuko ENOHARA,* Yoshiyuki* AMANO: Shokuhin Eiseigaku Zasshi (Journal of the Food Hygienic Soc. of Japan) 9, 139-146 (1968).

3. Toshio HAYASHI, Shokuhin Eisei Kenkyu (Studies in Food Hygiene), 18, 1050-1053 (1968).

4. Kunihiko MIYAMOTO, Tsuyoshi YOKOYAMA: Eika (abbreviated title; "Health Chemistry" ..) 22, 76-80 (1976).

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* Translator's note: reading not quite certain; other readings possible.

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